General Abstract **A Phase I Trial of Recombinant Vaccinia Viruses That Express DF3/MUC1 and TRICOM (B7.1, ICAM-1, and LFA-3) in Patients With Metastatic Adenocarcinoma of The Breast**

Interest in the immunologic rejection of malignant tumors was stimulated by the findings that mice could be immunized to reject chemically-induced tumors from genetically identical animals. Recombinant vaccinia virus containing sequences that encode antigens from infectious organisms can produce specific immune responses. Moreover, since vaccinia virus was used for years in vaccinations against smallpox, it has a well-known toxicity and safety profile.

Cell mediated immunity has been shown to require at least two signals. The first signal is the target (antigen) and the second is a co-stimulatory signal. Several molecules normally found on the surface of antigen presenting cells (APCs) have been shown to be capable of providing the second signal for cellular immune activation. Each of these molecules has been inserted into poxvirus vectors and each has been shown to efficiently mediate cell costimulation.

The DF3 (MUC1) mucin-like glycoprotein is particularly attractive as a potential target for cell mediated specific immunotherapy. Over 75% of breast carcinomas overexpress this glycoprotein. Moreover, vaccination with recombinant vaccinia virus expressing MUC1 prevented tumor development in animals challenged with tumors that express the antigen. Animal studies have demonstrated that treatment of established DF3/MUC1 positive pulmonary metastases with rV-DF3/MUC1 results in complete regressions. An admixture containing rV-DF3/MUC1 and recombinant vaccinia virus containing the co-stimulatory molecule B7.1 (rV-B7.1) inoculated into mice bearing a MUC1-expressing breast cancer demonstrated enhanced MUC1-specific anti-tumor responses which correlated with 100% survival. Murine dendritic cells infected with recombinant vaccinia or fowlpox vectors containing a triad of co-stimulatory molecules (B7.1, ICAM-1, and LFA-3, designated TRICOM) induced the activation of T-cell populations to far greater levels than those activated by one or two co-stimulatory molecules. There was no agent-related toxicity observed. rV-TRICOM has now become available for clinical trials.

A Phase I clinical trial of rV-DF3/MUC1 is underway at the DFCI. Nineteen patients have been treated. No clinical toxicities attributable to either the vaccinia vector or the DF3/MUC1 antigen have been observed. Two patients have demonstrated a decrease in serum CA 27-29 (the assay used to determine serum levels of DF3/MUC1 in clinical specimens at the DFCI), one for 6^{+} months and 2 other patients have demonstrated stable serum CA 27-29 for 1^{+} to 3^{+} months.

This is an open label Phase I study of rV-DF3/MUC1 and rV-TRICOM to patients with metastatic breast cancer. These vaccinations will be administered to each patient at 4 week intervals for a total of 2 doses unless there is evidence of disease progression or unacceptable toxicity. Five to ten will be treated at each dose level and evaluated for toxicity at 4 weeks after initial vaccination before entry of patients at the next higher dose level (at least 5 patients need a particular immune marker for the laboratory testing). Two different concentrations of rV-DF3/MUC1 and rV-TRICOM will be used for escalation. The starting dose represents ~0.05 or 5% of the dose used in the rV-DF3/MUC1 trial, which was not a maximum tolerated dose (MTD) but the maximum deliverable dose. If no dose limiting toxicity is encountered at this dose level, a subsequent group of 5-10 patients will be treated with an increased dose of rV-DF3/MUC1 and rV-TRICOM. If a dose limiting toxicity is encountered at any dose level, three additional patients will be added to that dose level. If two out of six patients present grade III toxicity at a given dose, then the next lower dose will be considered the MTD. Five to ten additional patients will be treated with the admixture and locally administered recombinant GM-CSF at 100 mcg at the maximum dose of the admixture. Since GM-CSF may have additional properties to enhance active specific immunity, we propose to add local subcutaneous injection of GM-CSF to intradermal administration of rV-DF3/MUC1/rV-TRICOM at the MTD of the viral vectors alone. Weekly after the first vaccination, patients will be evaluated for toxicity, including a directed history, physical examination, and immunological assays. Prior to subsequent vaccination, patients will be re-evaluated and a history and physical examination will be performed, including an assessment of current performance status, blood analysis, quality of life and any side effects or toxicity from the vaccine. If toxicity is present, appropriate medical intervention will be provided, including the possibility of withdrawal. Four weeks after the final vaccination the patient will return to the clinic for a final clinical evaluation.